REMARKS

The Examiner has rejected Claim 3 under 35 U.S.C. § 112, second paragraph, as having insufficient antecedent basis for the phrase cryptic epitopes. The Examiner has also rejected Claim 15 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The Examiner has further rejected claims 1-7, 15, and 21 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Examiner has also rejected claims 1-7, 15, and 21 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification commensurate in scope with theses claims. In addition, the Examiner has rejected claims 1-7, 15, and 21 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over Claim 1 of U.S. Patent No. 5,843,454, and Claim 1 of U.S. Patent No. 5,518,723.

The Examiner has also rejected claims 1-3, 5, 7, 15, and 21 under 35 U.S.C. § 102(b) as being anticipated by Hart T. et al. (Proc Natl Acad Sci U S A. 1991 Mar 15; 88(6):2189-93; Cited as ref # 12 in IDS 12/3/03) ("Hart").

Claims 8-14 and 16-20 have been withdrawn. Claims 1-21 are currently pending. The following remarks are considered by applicant to overcome each of the Examiner's outstanding rejections to current claims 1-7, 15, and 21. An early Notice of Allowance is therefore requested.

I. REJECTION OF CLAIM 15 UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

On page 2 of the current Office Action, the Examiner rejects Claim 3 under 35 U.S.C. § 112, second paragraph, as lacking antecedent basis for the term "cryptic epitopes". This rejection is respectfully traversed and believed overcome in view of the following discussion.

The portion of Claim 3 to which Examiner objects is the portion which states:

"...wherein **cryptic epitopes** are revealed." (emphasis added).

As can be seen, the term "cryptic epitopes" is plural, and not singular. It is grammatically incorrect to insert the word "a" or the word "an" before a plural phrase. In addition, Claim 3 does not state "the" cryptic epitopes, but rather just "cryptic epitopes". This is the proper way to supply antecedent basis for a plural term. Therefore, Applicants respectfully request the Examiner remove the rejection of Claim 1 under 35 U.S.C. § 112, second paragraph.

II. REJECTION OF CLAIM 15 UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

On page 3 of the current Office Action, the Examiner rejects Claim 15 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. This rejection is respectfully traversed and believed overcome in view of the following discussion.

The Examiner asserts that the enablement requirement has not been satisfied because undue experimentation is required to make and use the full scope of the invention. In particular, that Examiner asserts that undue experimentation is required because (1) there are no working examples which suggest the desired results of a vaccine which would raise neutralizing antibodies in humans and protect against HIV infection, (2) the nature of the invention involved the complex and incompletely understood area of immune responses important in HIV Disease, and (3) the state of the prior art shows that prior vaccines designed to produce neutralizing antibody responses against HIV infection have been largely ineffective for the intended purpose. However, this line of reasoning misunderstands the intended use of Claim 15.

Claim 15 is meant to be used to help develop a vaccine for HIV. The Examiner seems to be focusing on the effectiveness of Claim 15 at preventing all strains of HIV, however, this misses the point.

A vaccine may contain more than one substance which aids in its effectiveness. For example, the flu vaccine consists of multiple substances which together inoculate people against multiple strains of influenza. One of those substances alone would not provide the same protection against influenza. "Each year the influenza virus changes and different strains become

dominant." Wikipedia, The Free Encyclopedia, http://en.wikipedia.org/wiki/Flu_vaccine (visited on 6/26/07) (a copy of this article is attached as Appendix A). "Due to the high mutability of the virus a particular vaccine formulation usually only works for about a year." *Id.* Accordingly, the flu vaccine must be modified every year in order to provide the best protection against influenza. However, the vaccine does not make a person immune to all strains of influenza, but rather only immune to the strains that were covered in the particular flu vaccine the patient received. Each substance in the flu vaccine that helps to prevent infection of influenza has obvious utility and effectiveness, even though each substance alone cannot prevent all infuenza strains. Moreover, the flu vaccine itself also has obvious utility and effectiveness, even though it does not completely prevent you from being infected by every strain of influenza.

Claim 15 states, in part:

"A vaccine comprising:

"an immunogenically effective amount of a complex of gp120 covalently bonded to a fragment of CD4 or an equivalent thereof in a pharmaceutically acceptable medium ...."

Accordingly, Claim 15 does not limit itself to only the specified substances, but merely states that all the specified substances must be present. The test results contained in the current specification clearly illustrate that the composition of Claim 15 elicits a broad anti-HIV response and therefore has great utility and effectiveness in vaccine development and immunotherapy against HIV infection. Specification, P. 1, Lns. 26-30; P. 2, Lns. 1-2; P. 5, Lns. 23-31; P. 6, Lns. 1-11. See also Specification, Examples section, P. 12-31.

As discussed above, the flu vaccine consists of multiple substances which together inoculate people against multiple strains of influenza. One of those substances may only be effective against a single strain, or it may take a few of those substances working in tandem to be effective against a single strain. Even though one of those substances is not effective against all influenza strains, one of ordinary skill in the art would know how to combine one substance that assists in immunization from one influenza strain with other substances that assist in

immunization from other influenza strains, such that together the various substances create a flu vaccine with effectiveness against a sufficient number of strains of influenza that people will buy it.

Here though, the question is not whether the invention of Claim 15 is effective against a sufficient number of strains of HIV such that people will buy it, nor is the question whether the invention of Claim 15 is effective against all strains of HIV. Rather, the question is whether the specification has enabled one of ordinary skill in the art to practice the invention of Claim 15. The specification need not specify how to create a marketable vaccine. The specification need only provide information sufficient to inform one of ordinary skill in the art how to practice the invention for its recited utility. The specification has set forth that the invention of Claim 15 is useful in the development of HIV vaccines. The specification has also set forth how to use the invention of Claim 15 to assist in the creation of a vaccine. This is all the enablement that is required.

The crux of the Examiner's rejection is that:

> "It is known in the art that the quasispecies nature of HIV and the plasticity of the HIV-1 genome contribute to its ability to alter itself in every replication cycle, and thereby acquire mutations to escape immune pressures, contributes to the difficulties in producing a vaccine. Neither the specification nor prior art has provided sufficient teaching to show the asserted HIV vaccine strategy can overcome the extraordinary variability of HIV, resulting an HIV vaccine. Therefore, the instant specification does not 'provide information sufficient to inform one of ordinary skill in the art how to practice the invention for its recited utility'."
> Office Action (05/15/07), P. 3, ¶ 8.

Essentially, the Examiner asserts that the specification must teach a vaccine that works for every strain of HIV. However, as discussed above, this misses the point. The specification need not teach a vaccine for all of the strains of HIV. Rather, teaching a component that would be useful to include in a vaccine because it can prevent one strain of HIV is sufficient. It matters not that the prevented strain may mutate into a new strain that the component will no

longer be capable of preventing, so long as the component is capable of preventing that one strain.

The specification has shown that gp120-CD4 complexes can elicit a broadly neutralizing antibody response. Specification, P. 1, Lns. 26-30; P. 2, Lns. 1-2; P. 5, Lns. 23-31; P. 6, Lns. 1-11. See also Specification, Examples section, P. 12-31. Accordingly, such complexes would be useful in a vaccine. While such complexes alone may not be sufficient to prevent contraction of all strains of HIV, it is nonetheless undeniable that such complexes would be useful to include as part of the many components that comprise a vaccine. Therefore, the current application has definitely enabled a person of ordinary skill in the art to incorporate the components as set forth in Claim 15 into a vaccine.

In response, Examiner asserts that Claim 15 claims a "full" vaccine, not a "partial" vaccine, and that Claim 15 should therefore be examined to its full scope. Examiner then proceed to discuss the challenges in HIV vaccine development. However, Claim 15 does not specify that the vaccine claimed is a vaccine for HIV. Accordingly, Examiner seems to be improperly amending the language of Claim 15 to read "A vaccine for HIV comprising", when Claim 15 in fact reads simply "A vaccine comprising".

Accordingly, it is not required that the specification enable a vaccine for all HIV strains, nor is it required that the specification enable a vaccine for all diseases. Rather, it is sufficient that the technology regarding vaccines in general is known in the art (e.g., polio, flu, chicken pox, etc.), and that the specification has and the general state of the art has enabled a vaccine with the components of Claim 15.

By analogy, should a patent application disclose that it is useful to include airplane-like wings on a car in order to create a vertical lift force that would essentially reduce the force the car exerts on the ground, thereby reducing frictional resistance and increasing gas mileage, it would be completely appropriate to include a claim for "A car comprising: ... airplane-like wings...." The application need not enable a flying car, as the claim does not state "A car which flies comprising...." It is sufficient that it is known in the state of the art how to

make a car. The specification need only enable the addition of wings to a car, as that is all that is claimed.

Similarly, Claim 15 only states "A vaccine comprising...", not "A vaccine for HIV comprising...." It is known in the state of the art how to make vaccines. The current specification has shown that it would be useful to include the components of Claim 15 in a vaccine. Accordingly, the Application need not enable "A vaccine for HIV comprising". It is sufficient that the Application has enabled a vaccine with the components of Claim 15, as this is what is claimed.

Therefore, Applicants respectfully assert that the Examiner's rejection of Claim 15 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement, is improper and that Claim 15 is in allowable form.

III. REJECTION OF CLAIMS 1-7, 15, AND 21 UNDER 35 U.S.C. § 112, FIRST PARAGRAPH – SCOPE OF ENABLEMENT

On page 4 of the current Office Action, the Examiner rejects claims 1-7, 15, and 21 under 35 U.S.C. § 112, first paragraph, because the specification, while enabling for an immunogenic complex comprising gp120 bonded to a fragment of CD4, does not reasonably provide enablement for an immunogenic complex comprising gp120 bonded to a CD4 equivalent. This rejection is respectfully traversed and believed overcome in view of the following discussion.

Amended Claim 1 states:

"An immunogenic complex comprising:

"gp120 covalently bonded to a fragment of CD4 or an equivalent thereof;

> "wherein an equivalent of a fragment of CD4 is any molecule that mimics the conformation of any fragment of CD4 and which can bind to gp120."

The phrase "CD4 equivalent molecules" is described in the specification as including any molecule that mimics CD4 in conformation and/or induces a conformational change on HIV-1 gp120 that is similar to that induced by CD4. Examiner argues that, as a result of this description, neither the instant claims nor the specification provides specific structure description about an equivalent of CD4. However, this claim analysis does not properly identify to what an "equivalent thereof" refers. An "equivalent thereof", as used in Claim 1, refers to an equivalent of a fragment of CD4, not an equivalent of CD4 in its entirety.

To this end, the Application specifically states that:

> "An 'equivalent' of any fragment of CD4 as used herein includes any molecule that mimics the conformation of any fragment of CD4 and which can bind to gp120." Application (as filed), P. 10, Lns. 24-26.

The portion of the specification to which the Examiner cites discusses equivalents of entire CD4 molecules. To better clarify what is meant by an "equivalent thereof" in Claim 1, Claim 1 has been currently amended. Accordingly, Applicants assert that Claim 1 is now in allowable form.

In response, the Examiner asserts that the phrase "the conformation of any fragment of CD4" and the phrase "cryptic epitopes" are not conventional in the art or known to one of ordinary skill in the art. This, however, is incorrect.

Regarding the phrase "the conformation of any fragment of CD4", the only words the Examiner can possibly be asserting are not conventional are "conformation" and "fragment of CD4". However, it is known that proteins fold into one, or more, specific spatial conformations, driven by a number of noncovalent interactions such as hydrogen bonding, ionic interactions, Van der Waals' forces and hydrophobic packing. Wikipedia, The Free Encyclopedia, http://en.wikipedia.org/wiki/Protein_conformation (visited on 3/20/08) (a copy of this article is attached as Appendix B). The "conformation" of proteins is something taught in

undergraduate level organic chemistry, and is therefore certainly conventional in the art. Accordingly, it is respectfully asserted that one of ordinary skill in the art would fully understand what "conformation" means in the above phrase. In addition, a "fragment of CD4" is self explanatory, as it is a "fragment" of CD4. Thus, one of ordinary skill in the art would certainly be familiar with this phrase.

Since the both word "conformational" and the phrase "fragment of CD4" are conventional in the art, their combination to form the phrase "the conformation of any fragment of CD4" must certainly be conventional in the art. Therefore, Applicants respectfully request the Examiner withdraw the assertion that the phrase "the conformation of any fragment of CD4" is not conventional in the art.

Similarly the phrase "cryptic epitopes" is also conventional in the art. In particular, a "cryptic epitope", also known as a cryptotope, is known to be an antigenic site or epitope hidden in protein or virion because it is present on the surface subunits that become buried. Wikipedia, The Free Encyclopedia, http://en.wikipedia.org/wiki/Cryptotope (visited on 3/20/08) (a copy of this article is attached as Appendix C). Moreover, a Google search of the phrase "what are cryptic epitopes" yielded about 287,000 results. A copy of the first ten results is attached as Appendix D. Such a widely used phrase must be conventional in the art. Therefore, Applicants respectfully request the Examiner withdraw the assertion that the phrase "cryptic epitopes" is not conventional in the art.

Therefore, Applicants respectfully assert that Examiner's rejection of claims 1-7, 15, and 21 under 35 U.S.C. § 112, first paragraph, because the specification, while enabling for an immunogenic complex comprising gp120 bonded to a fragment of CD4, does not reasonably provide enablement for an immunogenic complex comprising gp120 bonded to a CD4 equivalent, is improper and that claims 1-7, 15, and 21 are in allowable form.

IV. REJECTION OF CLAIMS 1-7, 15, AND 21 UNDER 35 U.S.C. § 112, FIRST PARAGRAPH – WRITTEN DESCRIPTION

On page 5 of the current Office Action, the Examiner rejects claims 1-7, 15, and 21 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. This rejection is respectfully traversed and believed overcome in view of the following discussion.

As stated above, the Application specifically states that:

> "An 'equivalent' of any fragment of CD4 as used herein includes any molecule that mimics the conformation of any fragment of CD4 and which can bind to gp120." Application (as filed), P. 10, Lns. 24-26.

The portion of the specification to which the Examiner cites discusses equivalents of entire CD4 molecules. To better clarify what is meant by an "equivalent thereof" in Claim 1, Claim 1 has been currently amended as discussed above. Accordingly, Applicants assert that Claim 1 is now in allowable form.

In response, the Examiner asserts that the phrase "the conformation of any fragment of CD4" and the phrase "cryptic epitopes" are not conventional in the art or known to one of ordinary skill in the art. As discussed above, however, this is incorrect. Both the phrase "the conformation of any fragment of CD4" and the phrase "cryptic epitopes" are conventional in the art. Therefore, Applicants respectfully request the Examiner withdraw the assertion that these phrases are not conventional in the art.

In addition, Examiner asserts that the claims as amended are directed to a vaccine, and that the current specification does not enable an HIV vaccine.

Firstly, not all of the claims are directed to a vaccine. Accordingly, this assertion by the examiner alone is insufficient to support the above rejection of all of claims 1-7, 15, and 21. Secondly, as discussed above, claim 15 is directed only to a "vaccine", and not specifically and "HIV vaccine". Thus, as stated above, the application need not enable an "HIV" vaccine. Also as stated above, vaccines, in general, are enabled in the art. The specification has simply

added to that enablement, by describing the components in claim one which may be included in a vaccine. It is sufficient that the Application has enabled simply a vaccine with the components of Claim 15, as this is what is claimed.

Therefore, Applicants respectfully assert that Examiner's rejection of claims 1-7, 15, and 21 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement, is improper and that claims 1-7 are in allowable form.

V. REJECTION OF CLAIMS 1-7 ON THE GROUND OF NONSTATUTORY OBVIOUSNESS-TYPE DOUBLE PATENTING

On page 5 of the current Office Action, the Examiner rejects claims 1-7 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over Claim 1 of U.S. Patent No. 5,843,454, and Claim 1 of U.S. Patent No. 5,518,723. This rejection is respectfully traversed and believed overcome in view of the following discussion.

Applicants respectful assert that the above rejections can be eliminated by the filing of a terminal disclaimer and that such a disclaimer need not be filed until the claims are otherwise determined to be allowable by the Examiner. Accordingly, Applicants respectfully reserve the right to file a terminal disclaimer and assert that the 1-7 are otherwise in allowable form.

VI. REJECTION OF CLAIMS 1-3, 5, 7, 15, AND 21 UNDER 35 U.S.C. § 102(B) IN VIEW OF HART

On page 6 of the current Office Action, the Examiner rejects claims 1-3, 5, 7, 15, and 21 under 35 U.S.C. § 102(b) as being anticipated by Hart. This rejection is respectfully traversed and believed overcome in view of the following discussion.

Claims 13, 5, 7, and 21

Claim 1 states, in part:

> "gp120 **covalently** bonded to a fragment of CD4 or an equivalent thereof". (emphasis added).

As such, Claim 1 requires a chemical covalent bond. However, the bond disclosed in Hart is a physical non-covalent bond. See Hart, P. 2189, Abstract, Lns. 3-5. In particular, Hart discloses using physical non-covalent interactions between soluble CD4 and gp120 to induce release of gp120 from HIV-1 and HIV-1 infected cells. Hart, P. 2192, Discussion, ¶ 1, Lns. 1-2. This non-covalent interaction is very different from the covalent bond as stated in Claim 1.

In particular, the covalent bond of Claim 1 results in a configuration of gp120 that is changed for immunogenic purposes, enhancing the immunogenic properties of gp120. More specifically, the changed configuration of gp120 results in cryptic epitopes of gp120, which are hidden in the initial conformation of gp120, being exposed. Application, P. 2, Ln. 26 – P. 3, Ln. 6. The covalent bond between gp120 and a fragment of CD4 or an equivalent thereof essentially locks this new conformation of gp120, such that the now exposed cryptic epitopes are also locked in an exposed state. This enables antibodies to be created to this exposed cryptic epitope.

The non-covalent bond of Hart, however, is quite different. In particular, such a physical, non-covalent bond results in only a transient conformational change in gp120. This transient conformational change makes it very difficult to form antibodies from any cryptic epitopes, as they are only exposed for a brief period of time before reverting back to their typical hidden state.

As Hart fails to disclose he claimed covalent bond as stated in Claim 1, Applicants respectfully assert that examiner has failed to establish a prima facie case of anticipation of independent Claim 1, and corresponding claims 2 3, 5, and 7 as they are all dependant from Claim 1. Therefore, Applicants respectfully request the Examiner remove the rejection of claims 1-3, 5, 7, and 21 under 35 U.S.C. § 102(b) as being anticipated by Hart T. et al. (Proc Natl Acad Sci U S A. 1991 Mar 15; 88(6):2189-93; Cited as ref # 12 in IDS 12/3/03).

Claim 15

Claim 15 states, in part:

"an immunogenically effective amount of a complex of gp120 **covalently** bonded to a fragment of CD4 or an equivalent thereof in a pharmaceutically acceptable medium". (emphasis added).

Similarly to Claim 1, Claim 15 requires a chemical covalent bond. However, as discussed above, the bond disclosed in Hart is a physical non-covalent bond.

As such, Applicants respectfully assert that examiner has failed to establish a prima facie case of anticipation of independent Claim 15. Therefore, Applicants respectfully request the Examiner remove the rejection of Claim 15under 35 U.S.C. § 102(b) as being anticipated by Hart T. et al. (Proc Natl Acad Sci U S A. 1991 Mar 15; 88(6):2189-93; Cited as ref # 12 in IDS 12/3/03).

Based upon the above remarks, Applicant respectfully requests reconsideration of this application and its early allowance. Should the Examiner feel that a telephone conference with Applicant's attorney would expedite the prosecution of this application, the Examiner is urged to contact him at the number indicated below.

Respectfully submitted,

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ED:JWT

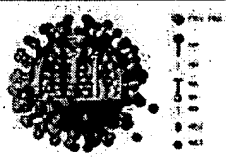
502615.20014

Appendix A

Flu vaccine

From Wikipedia, the free encyclopedia

Flu



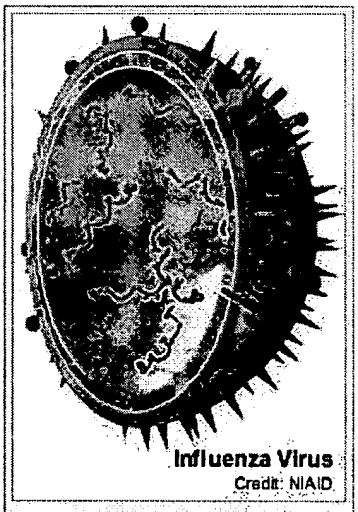
- Flu
- Flu season
- **Flu vaccine**
- Flu treatment
- Avian flu
- H5N1 flu
- Flu research
- Genome sequencing

The **flu vaccine** is a vaccine to protect against the highly variable influenza virus.

The annual flu kills an estimated 36,000 people in the United States each year. The annually updated trivalent flu vaccine for the 2006–2007 season consists of hemagglutinin (HA) surface glycoprotein components from influenza H3N2, H1N1, and B influenza viruses.[1]

Each year the influenza virus changes and different strains become dominant. Due to the high mutability of the virus a particular vaccine formulation usually only works for about a year. The World Health Organization co-ordinates the contents of the vaccine each year to contain the most likely strains of the virus to attack the next year. The flu vaccine is usually recommended for anyone in a high-risk group who would be likely to suffer complications from influenza.

Contents



Model of Influenza Virus from NIH

History of the flu vaccine

See also: Timeline of vaccines

Vaccines are used in both humans and nonhumans. Human vaccine is meant unless specifically identified as a veterinary or poultry or livestock vaccine.

Vaccines prior to flu vaccines

A vaccine is an antigenic preparation used to produce active immunity to a disease, in order to prevent or ameliorate the effects of infection by any natural or "wild" strain of the organism. The process of distributing and administrating vaccines is referred to as vaccination. Smallpox is the first disease people tried to prevent by purposely inoculating themselves with other types of infections. Smallpox inoculation is believed to have started in India or China before 200 BC.[2] In 1718, Lady Mary Wortley Montague reported that the Turks have a habit of deliberately inoculating themselves with fluid taken from mild cases of smallpox and she inoculated her own children. In 1796 Edward Jenner inoculated using cowpox (a mild relative of the deadly smallpox virus). Pasteur and others built on this.[3]

Influenza

Influenza, commonly known as the flu, is an infectious disease that infects birds and mammals (primarily of the upper airways and lungs in mammals) and is caused by an RNA virus of the Orthomyxoviridae family (the influenza viruses). The most common and characteristic symptoms of influenza in humans are fever, pharyngitis (sore throat), myalgia (muscle pains), severe headache, coughing, and malaise (weakness and fatigue).[4] Hippocrates first described the symptoms of influenza in 412 B.C. Since then, the virus has undergone mutations and shifts and has caused numerous pandemics. The first influenza pandemic was recorded in 1580, since this time, various methods have been employed to eradicate its cause.[5] The etiological cause of influenza, the orthomyxoviridae was finally discovered by the Medical Research Council (MRC) of the United Kingdom in 1933.[6]

Known flu pandemics:[7]

- 1889–90 - Asiatic (Russian) Flu, mortality rate said to be 0.75-1 death per 1000 possibly H2N2
- 1900 - possibly H3N8
- 1918–20 – Spanish Flu, 500 million ill, at least 40 million died of H1N1
- 1957–58 – Asian Flu, 1 to 1.5 million died of H2N2
- 1968–69 – Hong Kong Flu, 3/4 to 1 million died of H3N2

Flu vaccine origins

In the world wide Spanish flu pandemic of 1918, "Physicians tried everything they knew, everything they had ever heard of, from the ancient art of bleeding patients, to administering oxygen, to developing new vaccines and sera (chiefly against what we now call *Hemophilus influenzae*—a name derived from the fact that it was originally considered the etiological agent—and several types of pneumococci). Only one therapeutic measure, transfusing blood from recovered patients to new victims, showed any hint of success."[8]

"In 1931, viral growth in embryonated hens' eggs was discovered, and in the 1940s, the U.S. military developed the first approved inactivated vaccines for influenza, which were used in the Second World War".[3]

Flu vaccine acceptance

The current egg-based technology for producing influenza vaccine was created in the 1950s.[9]

"The WHO Global Influenza Surveillance Network was established in 1952. The network comprises 4 WHO Collaborating Centres (WHO CCs) and 112 institutions in 83 countries, which are recognized by WHO as WHO National Influenza Centres (NICs). These NICs collect specimens in their country, perform primary virus isolation and preliminary antigenic characterization. They ship newly isolated strains to WHO CCs for high level antigenic and genetic analysis, the result of which forms the basis for WHO recommendations on the composition of influenza vaccine for the Northern and Southern Hemisphere each year."[10]

In the U.S. swine flu scare of 1976 President Gerald Ford was confronted with a potential swine flu pandemic. The vaccination program was plagued by delays and public relations problems, but about 24% of the population was vaccinated by the time the program was canceled with much concern and doubt about flu vaccination.[11]

According to the CDC: "Influenza vaccination is the primary method for preventing influenza and its severe complications. [...] Vaccination is associated with reductions in influenza-related respiratory illness and physician visits among all age groups, hospitalization and death among persons at high risk, otitis media among children, and work absenteeism among adults. Although influenza vaccination levels increased substantially during the 1990s, further improvements in vaccine coverage levels are needed".[12]

Current status

"Vaccination in the veterinary world pursues four goals: (i) protection from clinical disease, (ii) protection from infection with virulent virus, (iii) protection from virus excretion, and (iv) serological differentiation of infected from vaccinated animals (so-called DIVA principle). In the field of influenza vaccination, neither commercially available nor experimentally tested vaccines have been shown so far to fulfil all of these requirements."[13]

Flu research includes molecular virology, molecular evolution, pathogenesis, host immune responses, genomics, and epidemiology. These help in developing influenza countermeasures

such as vaccines, therapies and diagnostic tools. Improved influenza countermeasures require basic research on how viruses enter cells, replicate, mutate, evolve into new strains and induce an immune response. The Influenza Genome Sequencing Project is creating a library of influenza sequences that will help us understand what makes one strain more lethal than another, what genetic determinants most affect immunogenicity, and how the virus evolves over time. Solutions to limitations in current vaccine methods are being researched.

"Today, we have the capability to produce 300 million doses of trivalent vaccine per year - enough for current epidemics in the Western world, but insufficient for coping with a pandemic."[3][14]

Clinical trials of vaccines

A vaccine is assessed in terms of the reduction of the risk of disease produced by vaccination, its *efficacy*. In contrast, in the field, the *effectiveness* of a vaccine is the practical reduction in risk for an individual when they are vaccinated under real-world conditions.[15] Measuring efficacy of influenza vaccines is relatively simple, as the immune response produced by the vaccine can be assessed in animal models, or the amount of antibody produced in vaccinated people can be measured,[16] or most rigorously, by immunising adult volunteers and then challenging with virulent influenza virus.[17] In studies such as these, influenza vaccines showed high efficacy and produced a protective immune response. For ethical reasons, such challenge studies cannot be performed in the population most at risk from influenza - the elderly and young children. However, studies on the effectiveness of flu vaccines in the real world are uniquely difficult. The vaccine may not be matched to the virus in circulation, virus prevalence varies widely between years and influenza is often confused with other flu-like illnesses.[18]

Nevertheless, multiple clinical trials of both live and inactivated influenza vaccines have been performed and their results pooled and analyzed in several recent meta-analyses. Studies on live vaccines have very limited data, but these preparations may be more effective than inactivated vaccines.[17] The meta-analyses examined the efficacy and effectiveness of inactivated vaccines in adults,[19] children,[20] and the elderly.[21][22] In adults, vaccines show high efficacy against the targeted strains, but low effectiveness overall, so the benefits of vaccination are small, with a one-quarter reduction in risk of contracting influenza but no effect on the rate of hospitalization.[19] In children, vaccines again showed high efficacy, but low effectiveness in preventing "flu-like illness", in children under two the data are extremely limited, but vaccination appeared to confer no measurable benefit.[20] In the elderly, vaccination does not reduce the frequency of influenza, but may reduce pneumonia, hospital admission and deaths from influenza or pneumonia.[21][22] The measured effectiveness of the vaccine in the elderly varies depending on whether the population studies is in residential care homes, or in the community, with the vaccine appearing more effective in an institutional environment. This apparent effect may be due to selection bias or differences in diagnosis and surveillance.

Overall, the benefit of influenza vaccination is clearest in the elderly, with vaccination in children of questionable benefit. Vaccination of adults is not predicted to produce significant improvements in public health. The apparent contradiction between vaccines with high efficacy,

but low effectiveness, may reflect the difficulty in diagnosing influenza under clinical conditions and the large number of strains circulating in the population.[18]

Who should get it


U.S. Navy personnel receiving influenza vaccination

Yearly influenza vaccination should be routinely offered to patients at risk of complications of influenza:

- the elderly (UK recommendation is those aged 65 or above)
- patients with chronic lung diseases (asthma, COPD, etc.)
- patients with chronic heart diseases (congenital heart disease, chronic heart failure, ischaemic heart disease)
- patients with chronic liver diseases (including liver cirrhosis)
- patients who are immunosuppressed (those with HIV or who are receiving drugs to suppress the immune system such as chemotherapy and long-term steroids) and their household contacts
- all people who are institutionalized in an environment where influenza can spread rapidly, such as in prisons or nursing homes
- healthcare workers (both to prevent sickness and to prevent spread to patients)[23]

The only contraindication is known anaphylaxis to the vaccine or its component.

In the United States a person aged 50–64 is nearly ten times more likely to die an influenza-associated death as a person under age 50 and a person over age 65 is over ten times more likely to die an influenza-associated death as a person in the 50–64 age group.[24] Vaccination of those over age 65 reduces influenza-associated death by about 50%.[25][26] However, it is unlikely that the vaccine completely explains the results since elderly people who get the vaccine are likely more healthy and health-conscious than those who do not.[27]

Additionally, because mortality is high among infants who contract influenza, the household contacts and caregivers of infants should get a flu vaccine to reduce the risk of passing an influenza infection to the infant.

Data from the years when Japan required annual flu vaccinations for school-aged children indicate that vaccinating children—the group most likely to catch and spread the disease—has a strikingly positive effect on reducing mortality among older people: one life saved for every 420 children who received the flu vaccine.[28] This may be due to herd immunity, or due to direct causes, such as individual older people not being exposed to influenza. For example, retired grandparents often care for their sick grandchildren in households where the parents can't take time off work or are sick themselves. The grandparents, who are at high risk for complications, hospitalization, and death, then catch the disease as a result of caring for infected younger

people.

Flu vaccine virus selection

Selecting viruses for the vaccine manufacturing process is very difficult.

At the U.S.'s Food and Drug Administration's (FDA) Center for Biologics Evaluation and Research's Vaccines and Related Biological Products Advisory Committee's 101st meeting of February 16, 2005, an extensive discussion and vote was held concerning next year's flu vaccine virus selection; but began with a summary of the previous year:

Influenza B

> "For Influenza B, the question was asked: are there new strains present? And the answer was yes, and in 2004, the majority of the viruses were similar to a strain called B/Shanghai/361/2002, which is from the so-called B/Yamagata/1688 hemagglutinin lineage. That lineage was not the one that was being used in the vaccine that was current last year. In a minority of the strains that were found during the epidemiologic studies were similar to the strain that was in the vaccine for last year, which was B/Hong Kong/330/2001, which belongs to the HA lineage that we represent with the strain B/Victoria/287. In answer to the question were these new viruses spreading, the answer, of course, is definitely yes. The Fujian-like viruses had become widespread around the world and were predominant everywhere, and these B/Shanghai-like strains at the time we were holding this meeting in February were predominant not only in North America and the United States, but also in Asia and Europe."

New viruses

> "Were the new viruses that were identified and spreading, were those inhibited by the current vaccines? And this question, as it sometimes is, was not a very definite no or yes. It was a little bit difficult to interpret, but it seemed like many of the A/Fujian-like viruses were not well inhibited by the current vaccines, although some of them were. For the B/Shanghai-like strains, of course, we've known for a long time that these two divergent hemagglutinin lineages are not that well inhibited one by the other, and as time has gone on and antigenic drift has occurred in these strains, that has become truer. Generally we also know that for the B/Yamagata-like strains and the B/Victoria-like strains, that very young children and people who haven't been immunologically primed, exposure to one of these does not seem to immediately give antibodies that cross-react with the other HA lineage."

Manufacturing issues

> "So were there strains that were suitable for manufacturing? And the answer was yes. Of course, we all know that for inactivated vaccines and for live attenuated vaccines manufacturing depends on having egg adapted strains, either the wild-type or reassortant, and in the case of the live vaccine, of course, it has to be a reassortant for the attenuation

phenotype. But there were A/Fujian-like strains that were available, and there was a high growth reassortant that was being used in manufacturing for the Southern Hemisphere already, the A/Wyoming/3/2003 X 147 reassortant. For the B strain, there were a number of wild-type isolates that seemed to be suitable for manufacturing, including B/Jilin/20/2003 and B/Jiangsu/10/2003, in addition to the B/Shanghai/361 strain itself."

Strains selected

"So based on that, the strains that were selected for this year include A/New Caledonia/20/99-like strain, which in this case really is A/New Caledonia/20/99. For the B/Shanghai/361/2002-like recommendation that was made, there were all three of these strains, B/Shanghai, B/Jilin, and B/Jiangsu. And for the A/Fujian/411/2002-like recommendation that was made and the A/Wyoming/3/2003 strain was chosen or is the one that has become widely used for vaccine preparation. Now, the implications of the strain selection were that preparation of the vaccines was on schedule throughout the year. All of the strains seemed to be typical and easy to adapt for manufacturing purposes, and going into the summer, the supply of vaccine was expected to match the demand predicted by previous years' experiences."

Unexpected difficulties

"But what happened was that we ended up with a vaccine shortage at the end of the summer, and just to try to put that into a little perspective, from January until August, manufacturing had been progressing on schedule even including these two new strains that were recommended for use in vaccines, and it was anticipated there were going to be about 100 million doses of vaccine from all of the manufacturers combined for this year. In August of 2004, Chiron notified regulatory authorities about a sterility issue and indicated that investigation to identify the cause and the implementation of corrections was underway, and at that time Chiron made a public announcement indicating that there would be a possible delay in distribution and possibly a reduction in the amount of vaccine that would be available. You also probably all know that in early October of 2004, the MHRA, the UK regulatory authority, announced that they were suspending Chiron's license to manufacture inactivated influenza vaccine for three months, and that was based on the issues that have previously been identified and were in investigation and correction by Chiron. Subsequently, over the next few weeks and certainly by November of 2004, it became clear after consultation between FDA and MHRA that the vaccine that Chiron had planned to make was not going to be available for us in the United States."

Response to unexpected difficulties

"In response to that, there were a number of things that happened within the Public Health Service, and I'll just very briefly indicate some of those. At FDA there was a lot of work done to evaluate manufacturers who were not licensed in the United States to identify whether their vaccines could be used under IND. There was consultation with manufacturers to discuss regulatory mechanisms going forward from this time for getting approval of new products in the United States. That includes accelerated approval, fast track and priority reviews to facilitate those new licenses, and all of these things actually

have been continuing."[29]

Flu vaccine manufacturing

Flu vaccines are available both as an injection of killed virus and as nasal spray of live attenuated influenza virus (LAIV) (sold as FluMist). Clinical trials suggest that the live virus may be more effective at preventing infection, but FluMist has not been approved in the United States for use in children younger than 5.[30]

Flu vaccine is usually grown in fertilized chicken eggs. Both types of flu vaccines are contraindicated for those with severe allergies to egg proteins and people with a history of Guillain-Barré syndrome.[31]

On October 5, 2004, Chiron Corporation, a corporation contracted to deliver half of the expected flu vaccine for the United States and a significant portion to the UK, issued a press release[32] that stated it was unable to dispense its stock for the 2004-2005 season, due to suspension of the corporation's license to produce the vaccine by the Medicines and Healthcare Products Regulatory Agency. However, the Centers for Disease Control and Prevention took swift action to enlist the help of other companies such as MedImmune and Sanofi pasteur to supply vaccine in high-risk populations in the United States.

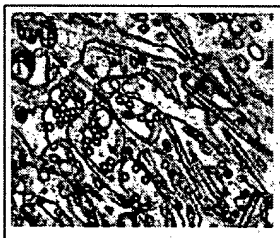
H5N1

There are several H5N1 vaccines for several of the avian H5N1 varieties, some for use in humans and some for use in poultry. H5N1 continually mutates, meaning vaccines based on current samples of avian H5N1 cannot be depended upon to work in the case of a future pandemic of H5N1. While there can be some cross-protection against related flu strains, the best protection would be from a vaccine specifically produced for any future pandemic flu virus strain. Dr. Daniel Lucey, co-director of the Biohazardous Threats and Emerging Diseases graduate program at Georgetown University has made this point, "There is no H5N1 pandemic so there can be no pandemic vaccine." However, "pre-pandemic vaccines" have been created; are being refined and tested; and do have some promise both in furthering research and preparedness for the next pandemic. Vaccine manufacturing companies are being encouraged to increase capacity so that if a pandemic vaccine is needed, facilities will be available for rapid production of large amounts of a vaccine specific to a new pandemic strain.

Problems with H5N1 vaccine production include:

- lack of overall production capacity

H5N1



- Influenza A virus subtype H5N1
- Genetic structure
- Infection
- Human mortality
- Global spread
  - in 2006
- Social impact
- Pandemic

WHO pandemic phases

- lack of surge production capacity (it is impractical to develop a system that depends on hundreds of millions of 11-day old specialized eggs on a standby basis)
- the pandemic H5N1 might be lethal to chickens

1. Low risk
2. New virus
3. **Self limiting**
4. Person to person
5. Epidemic exists
6. Pandemic exists

Cell culture (cell-based) manufacturing technology can be applied to influenza vaccines as they are with most viral vaccines and thereby solve the problems associated with creating flu vaccines using chicken eggs as is currently done.[33][34] The US government has purchased from Sanofi Pasteur and Chiron Corporation several million doses of vaccine meant to be used in case of an influenza pandemic of H5N1 avian influenza and is conducting clinical trials with these vaccines.[35] Researchers at the University of Pittsburgh have had success with a genetically engineered vaccine that took only a month to make and completely protected chickens from the highly pathogenic H5N1 virus.[36]

According to the United States Department of Health & Human Services:

> In addition to supporting basic research on cell-based influenza vaccine development, HHS is currently supporting a number of vaccine manufacturers in the advanced development of cell-based influenza vaccines with the goal of developing U.S.-licensed cell-based influenza vaccines produced in the United States. Dose-sparing technologies. Current U.S.-licensed vaccines stimulate an immune response based on the quantity of HA (hemagglutinin) antigen included in the dose. Methods to stimulate a strong immune response using less HA antigen are being studied in H5N1 and H9N2 vaccine trials. These include changing the mode of delivery from intramuscular to intradermal and the addition of immune-enhancing adjuvant to the vaccine formulation. Additionally, HHS is soliciting contract proposals from manufacturers of vaccines, adjuvants, and medical devices for the development and licensure of influenza vaccines that will provide dose-sparing alternative strategies.[37]

Chiron Corporation is now recertified and under contract with the National Institutes of Health to produce 8,000-10,000 investigational doses of Avian Flu (H5N1) vaccine. MedImmune and Aventis Pasteur are under similar contracts.[38] The United States government hopes to obtain enough vaccine in 2006 to treat 4 million people. However, it is unclear whether this vaccine would be effective against a hypothetical mutated strain that would be easily transmitted through human populations, and the shelflife of stockpiled doses has yet to be determined.[39]

The *New England Journal of Medicine* reported on March 30, 2006 on one of dozens of vaccine studies currently being conducted. The Treanor et al. study was on vaccine produced from the human isolate (A/Vietnam/1203/2004 H5N1) of a virulent clade 1 influenza A (H5N1) virus with the use of a plasmid rescue system, with only the hemagglutinin and neuraminidase genes expressed and administered without adjuvant. "The rest of the genes were derived from an avirulent egg-adapted influenza A/PR/8/34 strain. The hemagglutinin gene was further modified to replace six basic amino acids associated with high pathogenicity in birds at the cleavage site between hemagglutinin 1 and hemagglutinin 2. Immunogenicity was assessed by microneutralization and hemagglutination-inhibition assays with the use of the vaccine virus,

although a subgroup of samples were tested with the use of the wild-type influenza A/Vietnam/1203/2004 (H5N1) virus." The results of this study combined with others scheduled to be completed by Spring 2007 is hoped will provide a highly immunogenic vaccine that is cross-protective against heterologous influenza strains.[40]

On August 18, 2006. the World Health Organization changed the H5N1 strains recommended for candidate vaccines for the first time since 2004. "The WHO's new prototype strains, prepared by reverse genetics, include three new H5N1 subclades. The hemagglutinin sequences of most of the H5N1 avian influenza viruses circulating in the past few years fall into two genetic groups, or clades. Clade 1 includes human and bird isolates from Vietnam, Thailand, and Cambodia and bird isolates from Laos and Malaysia. Clade 2 viruses were first identified in bird isolates from China, Indonesia, Japan, and South Korea before spreading westward to the Middle East, Europe, and Africa. The clade 2 viruses have been primarily responsible for human H5N1 infections that have occurred during late 2005 and 2006, according to WHO. Genetic analysis has identified six subclades of clade 2, three of which have a distinct geographic distribution and have been implicated in human infections:

- Subclade 1, Indonesia
- Subclade 2, Middle East, Europe, and Africa
- Subclade 3, China

On the basis of the three subclades, the WHO is offering companies and other groups that are interested in pandemic vaccine development these three new prototype strains:

- An A/Indonesia/2/2005-like virus
- An A/Bar headed goose/Quinghai/1A/2005-like virus
- An A/Anhui/1/2005-like virus

[...] Until now, researchers have been working on prepandemic vaccines for H5N1 viruses in clade 1. In March, the first clinical trial of a U.S. vaccine for H5N1 showed modest results. In May, French researchers showed somewhat better results in a clinical trial of an H5N1 vaccine that included an adjuvant. Vaccine experts aren't sure if a vaccine effective against known H5N1 viral strains would be effective against future strains. Although the new viruses will now be available for vaccine research, WHO said clinical trials using the clade 1 viruses should continue as an essential step in pandemic preparedness, because the trials yield useful information on priming, cross-reactivity, and cross-protection by vaccine viruses from different clades and subclades."[41][42]

As of November 2006, the United States Department of Health and Human Services still had enough H5N1 pre-pandemic vaccine to treat about 3 million people (5.9 million full-potency doses) in spite of 0.2 million doses used for research and 1.4 million doses that have begun to lose potency (from the original 7.5 million full-potency doses purchased from Sanofi Pasteur and Chiron Corp.). The expected shelf life of seasonal flu vaccine is about a year so the fact that most of the H5N1 pre-pandemic stockpile is still good after about 2 years is considered encouraging. [43]

Flu seasons

2003–2004 season

The production of flu vaccine requires a lead time of about six months before the season. It is possible that by flu season a strain becomes common for which the vaccine does not provide protection. In the 2003-2004 season the vaccine was produced to protect against A/Panama, A/New Caledonia, and B/Hong Kong. A new strain, A/Fujian, was discovered after production of the vaccine started and vaccination gave only partial protection against this strain.

Nature magazine reported that the Influenza Genome Sequencing Project, using phylogenetic analysis of 156 H3N2 genomes, "explains the appearance, during the 2003–2004 season, of the 'Fujian/411/2002'-like strain, for which the existing vaccine had limited effectiveness" as due to an epidemiologically significant reassortment. "Through a reassortment event, a minor clade provided the haemagglutinin gene that later became part of the dominant strain after the 2002–2003 season. Two of our samples, A/New York/269/2003 (H3N2) and A/New York/32/2003 (H3N2), show that this minor clade continued to circulate in the 2003–2004 season, when most other isolates were reassortants."[44]

According to the CDC:

> During the 2003–2004 influenza season, influenza A (H1), A (H3N2), and B viruses co-circulated worldwide, and influenza A (H3N2) viruses predominated. Several Asian countries reported widespread outbreaks of avian influenza A (H5N1) among poultry. In Vietnam and Thailand, these outbreaks were associated with severe illnesses and deaths among humans. In the United States, the 2003-2004 influenza season began earlier than most seasons, peaked in December, was moderately severe in terms of its impact on mortality, and was associated predominantly with influenza A (H3N2) viruses.[45]

During September 28, 2003–May 22, 2004, WHO and NREVSS collaborating laboratories in the United States tested 130,577 respiratory specimens for influenza viruses; 24,649 (18.9%) were positive. Of these, 24,393 (99.0%) were influenza A viruses, and 249 (1.0%) were influenza B viruses. Among the influenza A viruses, 7,191 (29.5%) were subtyped; 7,189 (99.9%) were influenza A (H3N2) viruses, and two (0.1%) were influenza A (H1) viruses. The proportion of specimens testing positive for influenza first increased to >10% during the week ending October 25, 2003 (week 43), peaked at 35.2% during the week ending November 29 (week 48), and declined to <10% during the week ending January 17, 2004 (week 2). The peak percentage of specimens testing positive for influenza during the previous four seasons had ranged from 23% to 31% and peaked during late December to late February.[45]

As of June 15, 2004, CDC had antigenically characterized 1,024 influenza viruses collected by U.S. laboratories since October 1, 2003: 949 influenza A (H3N2) viruses, three influenza A (H1) viruses, one influenza A (H7N2) virus, and 71 influenza B viruses. Of the 949 influenza A (H3N2) isolates characterized, 106 (11.2%) were similar antigenically to the vaccine strain

A/Panama/2007/99 (H3N2), and 843 (88.8%) were similar to the drift variant, A/Fujian/411/2002 (H3N2). Of the three A (H1) isolates that were characterized, two were H1N1 viruses, and one was an H1N2 virus. The hemagglutinin proteins of the influenza A (H1) viruses were similar antigenically to the hemagglutinin of the vaccine strain A/New Caledonia/20/99. Of the 71 influenza B isolates that were characterized, 66 (93%) belonged to the B/Yamagata/16/88 lineage and were similar antigenically to B/Sichuan/379/99, and five (7%) belonged to the B/Victoria/2/87 lineage and were similar antigenically to the corresponding vaccine strain B/Hong Kong/330/2001.[45]

H9N2

In December 2003, one confirmed case of avian influenza A (H9N2) virus infection was reported in a child aged 5 years in Hong Kong. The child had fever, cough, and nasal discharge in late November, was hospitalized for 2 days, and fully recovered. The source of this child's H9N2 infection is unknown.[45]

H5N1

During January–March 2004, a total of 34 confirmed human cases of avian influenza A (H5N1) virus infection were reported in Vietnam and Thailand. The cases were associated with severe respiratory illness requiring hospitalization and a case-fatality proportion of 68% (Vietnam: 22 cases, 15 deaths; Thailand: 12 cases, eight deaths). A substantial proportion of the cases were among children and young adults (i.e., persons aged 5–24 years). These cases were associated with widespread outbreaks of highly pathogenic H5N1 influenza among domestic poultry.[45]

H7N3

During March 2004, health authorities in Canada reported two confirmed cases of avian influenza A (H7N3) virus infection in poultry workers who were involved in culling of poultry during outbreaks of highly pathogenic H7N3 on farms in the Fraser River Valley, British Columbia. One patient had unilateral conjunctivitis and nasal discharge, and the other had unilateral conjunctivitis and headache. Both illnesses resolved without hospitalization.[45]

H7N2

During the 2003–2004 influenza season, a case of avian influenza A (H7N2) virus infection was detected in an adult male from New York, who was hospitalized for upper and lower respiratory tract illness in November 2003. Influenza A (H7N2) virus was isolated from a respiratory specimen from the patient, whose acute symptoms resolved. The source of this person's infection is unknown.[45]

2004–2005 season

According to the CDC:

> On the basis of antigenic analyses of recently isolated influenza viruses, epidemiologic data, and postvaccination serologic studies in humans, the Food and Drug Administration's Vaccines and Related Biological Products Advisory Committee (VRBPAC) recommended that the 2004–05 trivalent influenza vaccine for the United States contain A/New Caledonia/20/99-like (H1N1), A/Fujian/411/2002-like (H3N2), and B/Shanghai/361/2002-like viruses. Because of the growth properties of the A/Wyoming/3/2003 and B/Jiangsu/10/2003 viruses, U.S. vaccine manufacturers are using these antigenically equivalent strains in the vaccine as the H3N2 and B components, respectively. The A/New Caledonia/20/99 virus will be retained as the H1N1 component of the vaccine.[45]

2005–2006 season

The vaccines produced for the 2005–2006 season use:

- an A/New Caledonia/20/1999-like(H1N1);
- an A/California/7/2004-like(H3N2) (or the antigenically equivalent strain A/New York/55/2004);
- a B/Jiangsu/10/2003-like viruses.

In people in the U.S., overall flu and pneumonia deaths were below those of a typical flu season with 84% Influenzavirus A and the rest Influenzavirus B. Of the patients who had Type A viruses, 80% had viruses identical or similar to the A bugs in the vaccine. 70% of the people testing positive for a B virus had Type B Victoria, a version not found in the vaccine.[46]

"During the 2005–06 season, influenza A (H3N2) viruses predominated overall, but late in the season influenza B viruses were more frequently isolated than influenza A viruses. Influenza A (H1N1) viruses circulated at low levels throughout the season. Nationally, activity was low from October through early January, increased during February, and peaked in early March. Peak activity was less intense, but activity remained elevated for a longer period of time this season compared to the previous three seasons. The longer period of elevated activity may be due in part to regional differences in the timing of peak activity and intensity of influenza B activity later in the season."[47]

2006–2007 season

The 2006–07 influenza vaccine composition recommended by the World Health Organization on February 15, 2006 and the U.S. FDA's Vaccines and Related Biological Products Advisory Committee (VRBPAC) on February 17, 2006 use:

- an A/New Caledonia/20/99 (H1N1)-like virus;
- an A/Wisconsin/67/2005 (H3N2)-like virus (A/Wisconsin/67/2005 and A/Hiroshima/52/2005 strains);
- a B/Malaysia/2506/2004-like virus from B/Malaysia/2506/2004 and B/Ohio/1/2005 strains which are of B/Victoria/2/87 lineage.[48]

Flu vaccine for nonhumans

Horses with horse flu can run a fever, have a dry hacking cough, have a runny nose, and become depressed and reluctant to eat or drink for several days but usually recover in 2 to 3 weeks. "Vaccination schedules generally require a primary course of 2 doses, 3–6 weeks apart, followed by boosters at 6–12 month intervals. It is generally recognised that in many cases such schedules may not maintain protective levels of antibody and more frequent administration is advised in high-risk situations."[49]

"[P]oultry vaccines, made on the cheap, are not filtered and purified [like human vaccines] to remove bits of bacteria or other viruses. They usually contain whole virus, not just the hemagglutin spike that attaches to cells. Purification is far more expensive than the work in eggs, Dr. Stöhr said; a modest factory for human vaccine costs $100 million, and no veterinary manufacturer is ready to build one. Also, poultry vaccines are "adjuvated" — boosted — with mineral oil, which induces a strong immune reaction but can cause inflammation and abscesses. Chicken vaccinators who have accidentally jabbed themselves have developed painful swollen fingers or even lost thumbs, doctors said. Effectiveness may also be limited. Chicken vaccines are often only vaguely similar to circulating flu strains — some contain an H5N2 strain isolated in Mexico years ago. 'With a chicken, if you use a vaccine that's only 85 percent related, you'll get protection,' Dr. Cardona said. 'In humans, you can get a single point mutation, and a vaccine that's 99.99 percent related won't protect you.' And they are weaker [than human vaccines]. 'Chickens are smaller and you only need to protect them for six weeks, because that's how long they live till you eat them,' said Dr. John J. Treanor, a vaccine expert at the University of Rochester. Human seasonal flu vaccines contain about 45 micrograms of antigen, while an experimental A(H5N1) vaccine contains 180. Chicken vaccines may contain less than 1 microgram. 'You have to be careful about extrapolating data from poultry to humans,' warned Dr. David E. Swayne, director of the agriculture department's Southeast Poultry Research Laboratory. 'Birds are more closely related to dinosaurs.'"[50]

Researchers, led by Nicholas Savill of the University of Edinburgh in Scotland, used mathematical models to simulate the spread of H5N1 and concluded that "at least 95 per cent of birds need to be protected to prevent the virus spreading silently. In practice, it is difficult to protect more than 90 per cent of a flock; protection levels achieved by a vaccine are usually much lower than this."[51]

See also

- Vaccine for information valid about all vaccines, not just flu vaccines.
- Vaccine controversy for the pros and cons of being vaccinated.
- Thimerosal is a controversial mercury-containing organic compound used as an antiseptic and antifungal agent in vaccines.

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28. ^ http://content.nejm.org/cgi/content/abstract/344/12/889
29. ^ (transcript of U.S. FDA Center for Biologics Evaluation and Research Vaccines and Related Biological Products Advisory Committee's 101st meeting of February 16, 2005 is here: origin.www.fda.gov in .DOC format (http://origin.www.fda.gov/ohrms/dockets/ac/05/transcripts/2005-4087T1.DOC) in Google provided HTML format (http://www.google.com/search?q=cache:8hPXwU4pfyAJ:origin.www.fda.gov/ohrms/dockets/ac/05/transcripts/2005-4087T1.DOC+CDC+H5N1+research+%22two+clades%22&hl=en&gl=us&ct=clnk&cd=18))
30. ^ Yahoo News (http://news.yahoo.com/s/ap/20060501/ap_on_he_me/flu_spray)
31. ^ CDC (http://www.cdc.gov/flu/about/qa/flushot.htm)
32. ^ Chiron (http://www.chiron.com/investors/pressreleases/press_release100504.pdf)
33. ^ According to the U.S. HHS (United States Department of Health & Human Services) Pandemic Influenza Plan Appendix F: Current HHS Activities last revised on November 8, 2005 at http://www.hhs.gov/pandemicflu/plan/appendixf.html :

    > Currently, influenza vaccine for the annual, seasonal influenza program comes from four manufacturers. However, only a single manufacturer produces the annual vaccine entirely within the U.S. Thus, if a pandemic occurred and existing U.S.-based influenza vaccine manufacturing capacity was completely diverted to producing a pandemic vaccine, supply would be severely limited. Moreover, because the annual influenza manufacturing process takes place during most of the year, the time and capacity to produce vaccine against potential

pandemic viruses for a stockpile, while continuing annual influenza vaccine production, is limited. Since supply will be limited, it is critical for HHS to be able to direct vaccine distribution in accordance with predefined groups (see Appendix D (http://www.hhs.gov/pandemicflu/plan/appendixd.html)); HHS will ensure the building of capacity and will engage states in a discussion about the purchase and distribution of pandemic influenza vaccine.

Vaccine production capacity: The protective immune response generated by current influenza vaccines is largely based on viral hemagglutinin (HA) and neuraminidase (NA) antigens in the vaccine. As a consequence, the basis of influenza vaccine manufacturing is growing massive quantities of virus in order to have sufficient amounts of these protein antigens to stimulate immune responses. Influenza vaccines used in the United States and around world are manufactured by growing virus in fertilized hens' eggs, a commercial process that has been in place for decades. To achieve current vaccine production targets millions of 11-day old fertilized eggs must be available every day of production.

In the near term, further expansion of these systems will provide additional capacity for the U.S.-based production of both seasonal and pandemic vaccines, however, the surge capacity that will be needed for a pandemic response cannot be met by egg-based vaccine production alone, as it is impractical to develop a system that depends on hundreds of millions of 11-day old specialized eggs on a standby basis. In addition, because a pandemic could result from an avian influenza strain that is lethal to chickens, it is impossible to ensure that eggs will be available to produce vaccine when needed.

In contrast, cell culture manufacturing technology can be applied to influenza vaccines as they are with most viral vaccines (e.g., polio vaccine, measles-mumps-rubella vaccine, chickenpox vaccine). In this system, viruses are grown in closed systems such as bioreactors containing large numbers of cells in growth media rather than eggs. The surge capacity afforded by cell-based technology is insensitive to seasons and can be adjusted to vaccine demand, as capacity can be increased or decreased by the number of bioreactors or the volume used within a bioreactor. In addition to supporting basic research on cell-based influenza vaccine development, HHS is currently supporting a number of vaccine manufacturers in the advanced development of cell-based influenza vaccines with the goal of developing U.S.-licensed cell-based influenza vaccines produced in the United States.

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35. ^ New York Times article ""Doubt Cast on Stockpile of a Vaccine for Bird Flu"" (http://www.nytimes.com/2006/03/30/health/30vaccine.html?_r=1&oref=slogin) by Denise Grady. Published: March 30, 2006. Accessed 19 Oct 06
36. ^ Wired News (http://wired.com/news/wireservice/0,70102-0.html?tw=wn_index_7) JVI (http://jvi.asm.org/cgi/content/abstract/80/4/1959)
37. ^ Department of Health & Human Services (http://www.hhs.gov/pandemicflu/plan/appendixf.html)
38. ^ NAID - 2004 News (http://www3.niaid.nih.gov/news/newsreleases/2004/flucontracts.htm) NAID - 2005 News (http://www3.niaid.nih.gov/news/newsreleases/2005/medimmune.htm)
39. ^ NPR (http://www.npr.org/templates/story/story.php?storyId=5133306)
40. ^ New England Journal of Medicine (http://content.nejm.org/cgi/content/full/354/13/1411)Volume 354:1411-1413 - March 30, 2006 - Number 13 - *Vaccines against Avian Influenza — A Race against Time*
41. ^ CIDRAP News (http://www.cidrap.umn.edu/cidrap/content/influenza/avianflu/news/aug1806vaccines.html) article WHO changes H5N1 strains for pandemic vaccines, raising concern over virus evolution *published August 18, 2006*
42. ^ WHO (PDF} (http://www.who.int/csr/disease/avian_influenza/guidelines/recommendationvaccine.pdf) article *Antigenic and genetic characteristics of H5N1 viruses and candidate H5N1 vaccine viruses*

developed for potential use as pre-pandemic vaccines published August 18, 2006
43. ^ CIDRAP (http://www.cidrap.umn.edu/cidrap/content/influenza/panflu/news/nov1706stockpile.html) article *HHS: Most H5N1 vaccine on hand is still potent* published November 17, 2006
44. ^ Nature (http://www.nature.com/nature/journal/v437/n7062/full/nature04239.html)
45. ^ *a b c d e f g h* CDC (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5325a1.htm) article *Update: Influenza Activity --- United States and Worldwide, 2003—04 Season, and Composition of the 2004—05 Influenza Vaccine* published July 2, 2004
46. ^ Yahoo News (http://news.yahoo.com/s/ap/20060428/ap_on_he_me/flu_season;_ylt=ArqgzESNaMQox6OHu5Yxx-) Associated Press article *CDC: Milder Than Normal Flu Season Ending* published April 27, 2006
47. ^ CDC - 2005-06 U.S. INFLUENZA SEASON SUMMARY (http://www.cdc.gov/flu/weekly/weeklyarchives2005-2006/05-06summary.htm)
48. ^ CDC (http://www.cdc.gov/flu/professionals/vaccination/composition0607.htm) fluwatch (http://www.phac-aspc.gc.ca/fluwatch/05-06/w08_06/index.html) B/Victoria/2/87 lineage
49. ^ equiflunet_vaccines (http://195.224.162.216/equiflunet/equiflunet_vaccines.html)
50. ^ New York Times (http://www.nytimes.com/2006/05/02/science/02chic.html) article *Turning to Chickens in Fight With Bird Flu* published May 2, 2006
51. ^ SciDev.Net (http://www.scidev.net/gateways/index.cfm?fuseaction=readitem&rgwid=4&item=News&itemid=3051&language=1) article *Bird flu warning over partial protection of flocks* published August 16,2006

Further reading

- Read Congressional Research Service (CRS) Reports regarding Influenza and vaccines (http://digital.library.unt.edu/govdocs/crs/search.tkl?q=influenza&search_crit=subject&search=Search&date1=Anytime&date2=Anytime&type
- What's in a Flu Shot? (http://video.google.com/videoplay?docid=2163175142473074044)

Retrieved from "http://en.wikipedia.org/wiki/Flu_vaccine"

Categories: Influenza | Prevention | Vaccines

Appendix B

Protein structure

From Wikipedia, the free encyclopedia

Proteins are an important class of biological macromolecules present in all biological organisms, made up of such elements as carbon, hydrogen, nitrogen, phosphorus, oxygen, and sulfur. All proteins are polymers of amino acids. The polymers, also known as polypeptides consist of a sequence of 20 different L-α-amino acids, also referred to as residues. For chains under 40 residues the term peptide is frequently used instead of protein. To be able to perform their biological function, proteins fold into one, or more, specific spatial conformations, driven by a number of noncovalent interactions such as hydrogen bonding, ionic interactions, Van der Waals' forces and hydrophobic packing. In order to understand the functions of proteins at a molecular level, it is often necessary to determine the three dimensional structure of proteins. This is the topic of the scientific field of structural biology, that employs techniques such as X-ray crystallography or NMR spectroscopy, to determine the structure of proteins.

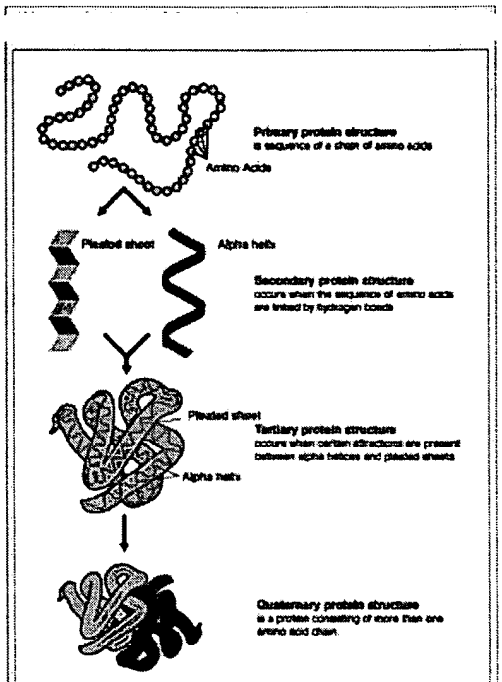
A number of residues are necessary to perform a particular biochemical function, and around 40-50 residues appears to be the lower limit for a functional domain size. Protein sizes range from this lower limit to several thousand residues in multi-functional or structural proteins. However, the current estimate for the average protein length is around 300 residues. Very large aggregates can be formed from protein subunits, for example many thousand actin molecules assemble into a collagen filament.

Contents

Levels of protein structure

Biochemistry refers to four distinct aspects of a protein's structure:

- **Primary structure** - the amino acid sequence of the peptide chains.
- **Secondary structure** - highly regular sub-structures (*alpha helix* and *strands of beta sheet*) which are locally defined, meaning that there can be many different secondary motifs present in one single protein molecule.
- **Tertiary structure** - Three-dimensional structure of a single protein molecule; a spatial arrangement of the secondary structures.
- **Quaternary structure** - complex of several protein molecules or polypeptide chains, usually called protein subunits in this context, which function as part of the larger assembly or protein complex.



Protein structure, from primary to quaternary structure.

In addition to these levels of structure, a protein may shift between several similar structures in performing its biological function. In the context of these functional rearrangements, these tertiary or quaternary structures are usually referred to as chemical conformation, and transitions between them are called conformational changes.

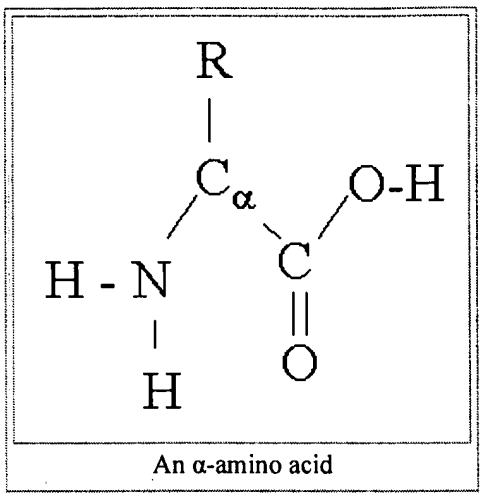
The primary structure is held together by covalent or peptide bonds, which are made during the process of protein biosynthesis or translation. These peptide bonds provide rigidity to the protein. The two ends of the amino acid chain are referred to as the C-terminal end or carboxyl terminus (C-terminus) and the N-terminal end or amino terminus (N-terminus) based on the nature of the free group on each extremity.

The various types of secondary structure are defined by their patterns of hydrogen bonds between the main-chain peptide groups. However, these hydrogen bonds are generally not stable by themselves, since the water-amide hydrogen bond is generally more favorable than the amide-amide hydrogen bond. Thus, secondary structure is stable only when the local concentration of water is sufficiently low, e.g., in the molten globule or fully folded states.

Similarly, the formation of molten globules and tertiary structure is driven mainly by structurally *non-specific* interactions, such as the rough propensities of the amino acids and hydrophobic interactions. However, the tertiary structure is *fixed* only when the parts of a protein domain are locked into place by structurally *specific* interactions, such as ionic interactions (salt bridges), hydrogen bonds and the tight packing of side chains. The tertiary structure of extracellular proteins can also be stabilized by disulfide bonds, which reduce the entropy of the unfolded state; disulfide bonds are extremely rare in cytosolic proteins, since the cytosol is generally a reducing environment.

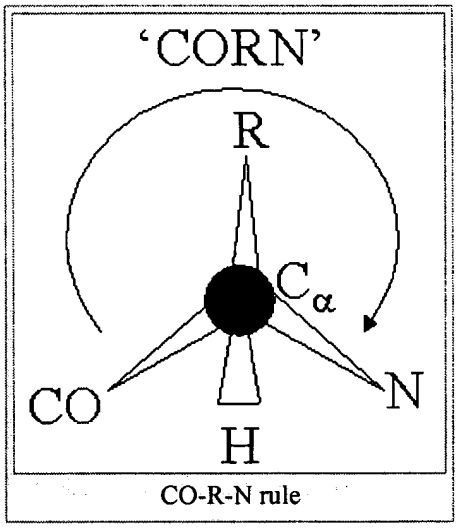
Structure of the amino acids

An α-amino acid consists of a part that is present in all the amino acid types, and a side chain that is unique to each type of residue. The C_α atom is bound to 4 different molecules (the H is omitted in the diagram); an amino group, a carboxyl group, a hydrogen and a side chain, specific for this type of amino acid. An exception from this rule is proline, where the hydrogen atom is replaced by a bond to the side chain. Because the carbon atom is bound to four different groups it is chiral, however only one of the isomers occur in biological proteins. Glycine however, is not chiral since its side chain is a hydrogen atom. A simple mnemonic for correct L-form is "CORN": when the C_α atom is viewed with the H in front, the residues read "CO-R-N" in a clockwise direction.



An α-amino acid

The side chain determines the chemical properties of the α-amino acid and may be any one of the 20 different side chains:

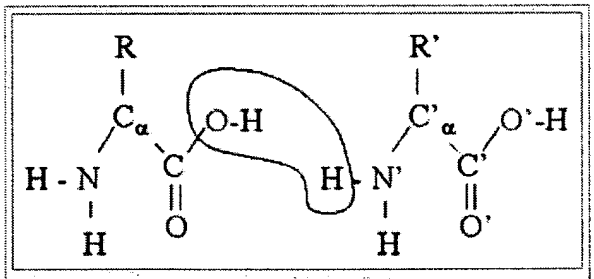


CO-R-N rule

Proline	PRO					90	
Tryptophan	TRP	W	1.0	186		163	P
Tyrosine	TYR	Y	2.2	163	10.1	141	P
Valine	VAL	V	6.0	99		105	H

The 20 naturally occurring amino acids can be divided into several groups based on their chemical proporties. Important factors are charge, hydrophobicity/hydrophilicity, size and functional groups. The nature of the interaction of the different side chains with the aqueous environment plays a major role in molding protein structure. Hydrophobic side chains tends to be buried in the middle of the protein, whereas hydrophilic side chains are exposed to the solvent. Examples of hydrophobic residues are: Leucine, isoleucine, phenylalanine, and valine, and to a lesser extent tyrosine, alanine and tryptophan. The charge of the side chains plays an important role in protein structures, since ion bonding can stabilize proteins structures, and an unpaired charge in the middle of a protein can disrupt structures. Charged residues are strongly hydrophilic, and are usually found on the out side of proteins. Positively charged side chains are found in lysine and arginine, and in some cases in histidine. Negative charges are found in glutamate and aspartate. The rest of the amino acids have smaller generally hydrophilic side chains with various functional groups. Serine and threonine have hydroxylgroups, and aspargine and glutamine have amide groups. Some amino acids have special properties such as cysteine, that can form covalent disulfide bonds to other cysteines, proline that is cyclical, and glycine that is small, and more flexible than the other amino acids.

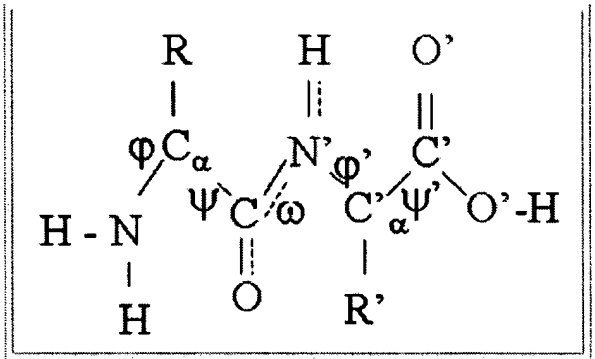
The peptide bond



Two amino acids

Two amino acids can be combined in a condensation reaction. By repeating this reaction, long chains of residues (amino acids in a peptide bond) can be generated. This reaction is catalysed by the ribosome in a process known as translation. The peptide bond is in fact planar due to the delocalization of the electrons from the double bond. The rigid peptide dihedral angle, ω (the bond between C_1 and N) is always close to 180 degrees. The dihedral angles φ (the bond between N and Cα) and psi ψ (the bond between Cα and C_1) can have a certain range of possible values. These angles are the degrees of freedom of a protein, they control the protein's three dimensional structure. They are restrained by geometry to allowed ranges typical for particular secondary structure elements, and represented in a

Ramachandran plot. A few important bond lengths are given in the table below.



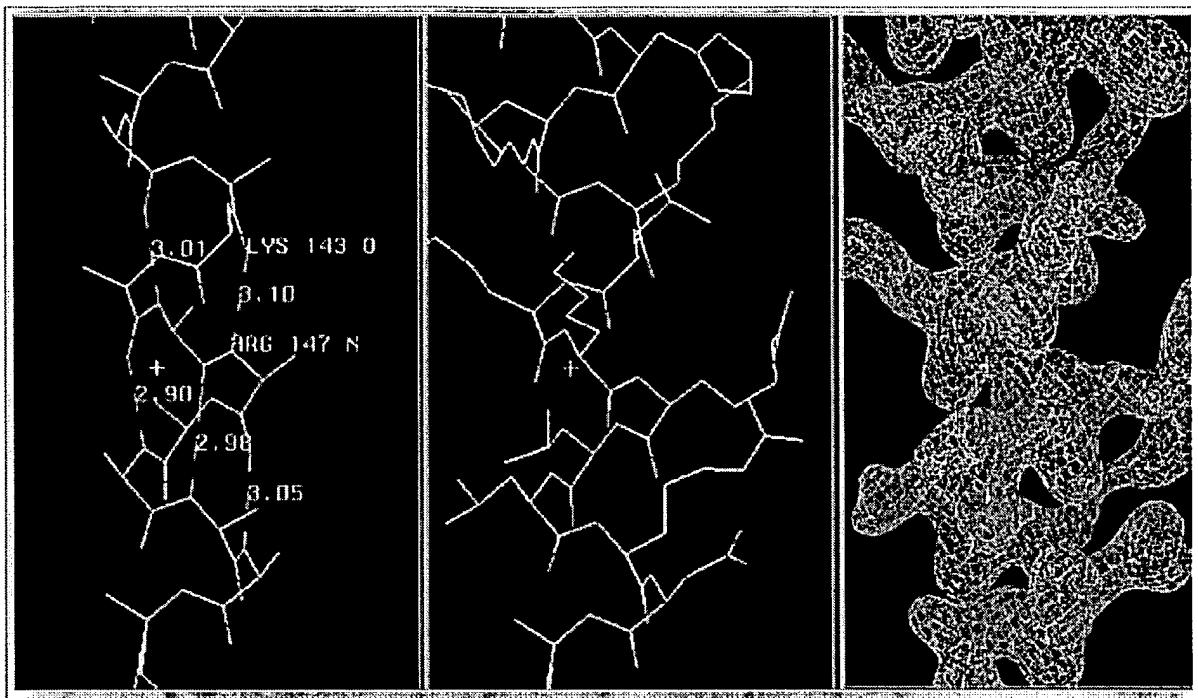
Bond angles for ψ and ω

Primary structure

The sequence of the different amino acids is called the primary structure of the peptide or protein. Counting of residues always starts at the N-terminal end (NH_2-group), which is the end where the amino group is not involved in a peptide bond. The primary structure of a protein is determined by the gene corresponding to the protein. A specific sequence of nucleotides in DNA is transcribed into mRNA, which is read by the ribosome in a process called translation. The sequence of a protein is unique to that protein, and defines the structure and function of the protein. The sequence of a protein can be determined by methods such as Edman degradation or tandem mass spectrometry. Often however, it is read directly from the sequence of the gene using the genetic code. Post-transcriptional modifications such as disulfide formation, phosphorylations and glycosylations are usually also considered a part of the primary structure, and cannot be read from the gene.

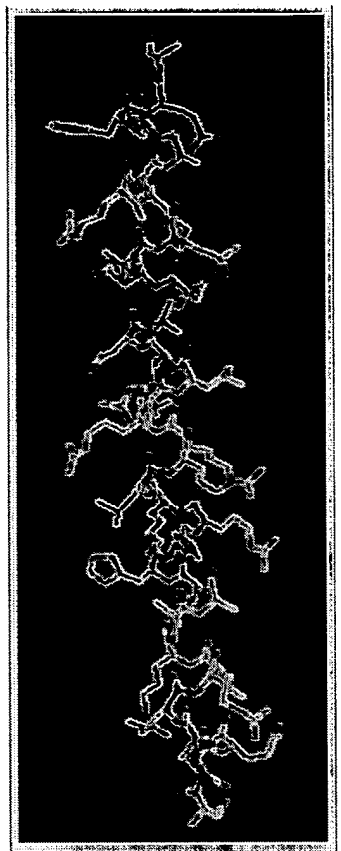
Secondary structure

By building models of peptides using known information about bond lengths and angles, the first elements of secondary structure, the alpha helix and the beta sheet, were suggested in 1951 by Linus Pauling and coworkers.[1] Both the alpha helix and the beta-sheet represent a way of saturating all the hydrogen bond donors and acceptors in the peptide backbone. These secondary structure elements only depend on properties that all the residues have in common, explaining why they occur frequently in most proteins. Since then other elements of secondary structure have been discovered such as various loops and other forms of helices. The part of the backbone that is not in a regular secondary structure is said to be random coil. Each of these two secondary structure elements have a regular geometry, meaning they are constrained to specific values of the dihedral angles ψ and φ. Thus they can be found in a specific region of the Ramachandran plot.

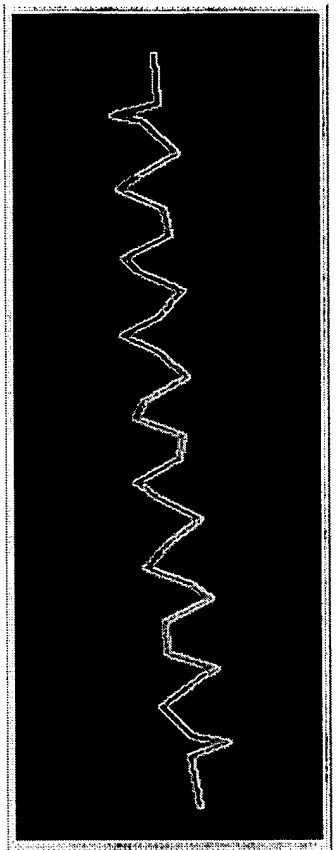


The left panel shows the hydrogen bonding in an actual α-helix backbone. Note that the *n*th residue O (Lys 1 bonds to the (*n*+4)th following residue's N (Arg 147). The actual values of some displayed H-bond distances you some idea about the variations to expect within a helix. The center panel includes the side chains which omitted in the left panel for clarity. You see the side chains pointing towards the N-terminal of the chain (lo residue numbers) and thus it is usually possible to determine the direction of the helix quite well during init model building. A 0.2 nm electron density is shown in the right panel

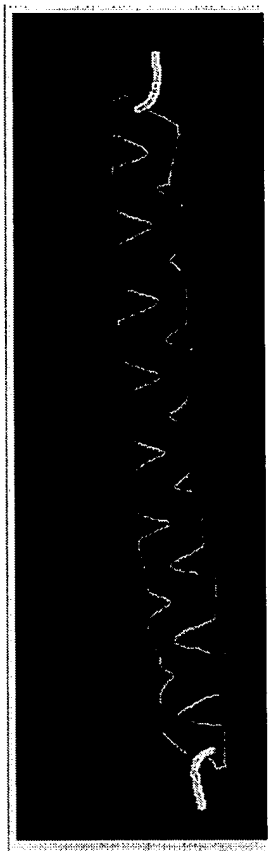
Here are some more representation of the same helix.



Ball and stick model



Backbone



Secondary structur
cartoon ("ribbon" o
"linguini diagram"

The hydrogen bond network in a 2-stranded, antiparallel β-sheet. The side chains are sticking out above or below the plane of the picture. It less clear cut than in the case of the helix, in which direction to initially trace a beta sheet strand. The beta sheet can be infinitely extended due to the repeatable H-bonding pattern to either side of a strand.

Turns, loops and a few other secondary structure elements such as a 3-10 helix complete the picture. We have now enough pieces to assemble a complete protein, displaying its typical tertiary structure.

Tertiary structure

The elements of secondary structure are usually folded into a compact shape using a variety of loops and turns. The formation of tertiary structure is usually driven by the burial of hydrophobic residues, but other interactions such as hydrogen bonding, ionic interactions and disulfide bonds

can also stabilize the tertiary structure. The tertiary structure encompasses all the noncovalent interactions that are not considered secondary structure, and is what defines the overall fold of the protein, and is usually indispensable for the function of the protein.

Quaternary structure

The quaternary structure is the interaction between several chains of peptide bonds. The individual chains are called subunits. The individual subunits are not necessarily covalently connected, but might be connected by a disulfide bond. Not all proteins have quaternary structure, since they might be functional as monomers. The quaternary structure is stabilized by the same range of interactions as the tertiary structure. Complexes of two or more polypeptides (i.e. multiple subunits) are called multimers. Specifically it would be called a dimer if it contains two subunits, a trimer if it contains three subunits, and a tetramer if it contains four subunits. Multimers made up of identical subunits may be referred to with a prefix of "homo-" (e.g. a homotetramer) and those made up of different subunits may be referred to with a prefix of "hetero-" (e.g. a heterodimer). Tertiary structures vary greatly from one protein to another. They are held together by glycosydic and covalent bonds.

Side chain conformation

The atoms along the side chain are named with Greek letters in Greek alphabetical order: α, β, γ, δ, ϵ and so on. C_α refers to the carbon atom closest to the carbonyl group of that amino acid, C_β the second closest and so on. The C_α is usually considered a part of the backbone. The dihedral angles around the bonds between these atoms are named $\chi 1$, $\chi 2$, $\chi 3$ etc. E.g. the first and second carbon atom in the side chain of lysine is named α and β, and the dihedral angle around the α-β bond is named $\chi 1$. Side chains can be in different conformations called gauche(-), trans and gauche(+). Side chains generally tend to try to come into a staggered conformation around $\chi 2$, driven by the minimization of the overlap between the electron orbitals of the hydrogen atoms.

Domains, motifs, and folds in protein structure

Many proteins are organized into several units. A structural domain is an element of the proteins overall structure that is self-stabilizing and often folds independently of the rest of the protein chain. Many domains are not unique to the protein products of one gene or one gene family but instead appear in a variety of proteins. Domains often are named and singled out because they figure prominently in the biological function of the protein they belong to; for example, the "calcium-binding domain of calmodulin". Because they are self-stabilizing, domains can be "swapped" by genetic engineering between one protein and another to make chimeras. A motif in this sense refers to a small specific combination of secondary structural elements (such as helix-turn-helix). These elements are often called supersecondary structures. Fold refers to a global type of arrangement, like helix-bundle or beta-barrel. Structure motifs usually consist of just a few elements, e.g. the 'helix-turn-helix' has just three. Note that while the *spatial sequence* of elements is the same in all instances of a motif, they may be encoded in any order within the underlying gene. Protein structural motifs often include loops of variable length and unspecified

structure, which in effect create the "slack" necessary to bring together in space two elements that are not encoded by immediately adjacent DNA sequences in a gene. Note also that even when two genes encode secondary structural elements of a motif in the same order, nevertheless they may specify somewhat different sequences of amino acids. This is true not only because of the complicated relationship between tertiary and primary structure, but because the size of the elements varies from one protein and the next. Despite the fact that there are about 100,000 different proteins expressed in eukaryotic systems, there are much fewer different domains, structural motifs and folds. This is partly a consequence of evolution, since genes or parts of genes can be doubled or moved around within the genome. This means that, for example, a protein domain might be moved from one protein to another thus giving the protein a new function. Because of these mechanisms pathways and mechanisms tends to be reused in several different proteins.

Protein folding

The process by which the higher structures form is called protein folding and is a consequence of the primary structure. A unique polypeptide may have more than one stable folded conformation, which could have a different biological activity, but usually, only one conformation is considered to be the active, or native conformation.

Structure classification

Several methods have been developed for the structural classification of proteins. These seek to classify the data in the Protein Data Bank in a structured order. Several databases exist which classify proteins using different methods. SCOP, CATH and FSSP are the largest ones. The methods used are purely manual, manual and automated, and purely automated. Work is being done to better integrate the current data. The classification is consistent between SCOP, CATH and FSSP for the majority of proteins which have been classified, but there are still some differences and inconsistencies.

Protein structure determination

Around 90% of the protein structures available in the Protein Data Bank have been determined by X-ray crystallography. This method allows one to measure the 3D density distribution of electrons in the protein (in the crystallized state) and thereby infer the 3D coordinates of all the atoms to be determined to a certain resolution. Roughly 9% of the known protein structures have been obtained by Nuclear Magnetic Resonance techniques, which can also be used to determine secondary structure. Note that aspects of the secondary structure as whole can be determined via other biochemical techniques such as circular dichroism. Secondary structure can also be predicted with a high degree of accuracy (see next section). Cryo-electron microscopy has recently become a means of determining protein structures to high resolution (less than 5 angstroms or 0.5 nanometer) and is anticipated to increase in power as a tool for high resolution work in the next decade. This technique is still a valuable resource for researchers working with very large protein complexes such as virus coat proteins and amyloid fibers.

A rough guide to the resolution of protein structures

Resolution	Meaning
>4.0	Individual coordinates meaningless
3.0 - 4.0	Fold possibly correct, but errors are very likely. Many sidechains placed with wrong rotamer.
2.5 - 3.0	Fold likely correct except that some surface loops might be mismodelled. Several long, thin sidechains (lys, glu, gln, etc) and small sidechains (ser, val, thr, etc) likely to have wrong rotamers.
2.0 - 2.5	As 2.5 - 3.0, but number of sidechains in wrong rotamer is considerably less. Many small errors can normally be detected. Fold normally correct and number of errors in surface loops is small. Water molecules and small ligands become visible.
1.5 - 2.0	Few residues have wrong rotamer. Many small errors can normally be detected. Folds are extremely rarely incorrect, even in surface loops.
0.5 - 1.5	In general, structures have almost no errors at this resolution. Rotamer libraries and geometry studies are made from these structures.

Computational prediction of protein structure

The generation of a protein sequence is much simpler than the generation of a protein structure. However, the structure of a protein gives much more insight in the function of the protein than its sequence. Therefore, a number of methods for the computational prediction of protein structure from its sequence have been proposed. *Ab initio* prediction methods use just the sequence of the protein. Threading uses existing protein structures.

Rosetta@home is a distributed computing project which tries to predict the structures of proteins with massive sampling on thousands of home computers.

Software

There are many available software packages, such as free web-based STING, used to visualize and analyze protein structures. Another example is the FeatureMap3D (http://www.cbs.dtu.dk/services/FeatureMap3D/) web-server which can visualize the quality of a protein-protein alignment in 3D and be used to map *sequence feature annotation* such as the underlying Intron/Exon structure onto a protein structure.

Several packages, such as Quantum Pharmaceuticals software[2], can be used to predict conformational changes of proteins and its influence on protein's functions.

Several methods have been developed to compare structures of different proteins. Please see structural alignment.

Computational tools are also frequently employed to check experimental and theoretical models of protein structures for errors (examples: ProSA (http://www.came.sbg.ac.at/typo3/index.php?

id=prosa), NQ-Flipper (https://flipper.services.came.sbg.ac.at/), Verify3D (http://www.doe-mbi.ucla.edu/Services/Verify_3D/), ANOLEA (http://www.swissmodel.unibas.ch/anolea/), WHAT_CHECK (http://swift.cmbi.ru.nl/gv/whatcheck/)).

References

1. ^ PAULING L, COREY RB, BRANSON HR. Proc Natl Acad Sci U S A. 1951 Apr;37(4):205-11. The structure of proteins; two hydrogen-bonded helical configurations of the polypeptide chain. PMID 14816373 (http://www.ncbi.nlm.nih.gov/pubmed/14816373)
2. ^ Quantum Pharmaceuticals software (http://www.q-pharm.com/)

Further reading

- Habeck M, Nilges M, Rieping W (2005). "Bayesian inference applied to macromolecular structure determination (http://www.spineurope.org/publications/Habeck%20et%20al%20031912%202005.pdf)". *Physical review. E, Statistical, nonlinear, and soft matter physics* 72 (3 Pt 1): 031912. PMID 16241487 (http://www.ncbi.nlm.nih.gov/pubmed/16241487).□ (Bayesian computational methods for the structure determination from NMR data)

External links

- ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php) Web service for the recognition of errors in experimentally or theoretically determined protein structures
- NQ-Flipper (https://flipper.services.came.sbg.ac.at/) Check for unfavorable rotamers of Asn and Gln residues in protein structures
- servers (http://swift.cmbi.ru.nl/) That check nearly 200 aspects of protein structure, like packing, geometry, unfavourable rotamers in general of for Asn, Gln and His especially, strange water molecules, backbone conformations, atom nomenclature, symmetry parameters, etc.
- Bioinformatics course (http://swift.cmbi.ru.nl/teach/B1/). An interactive, fully free, course explaining many of the aspects discussed in this wiki entry.

Appendix C

Cryptotope

From Wikipedia, the free encyclopedia

A **cryptotope** is an antigenic site or epitope hidden in protein or virion because it is present on the surface subunits that become buried. Cryptotopes are antigenically active only after disscociation of protein aggregates and virions[1] A cryptotope can also be referred to as a cryptic epitope. Cryptic epitopes or **cryptotopes** are becoming important for HIV vaccine research as a number of studies have shown that cryptic epitopes can be revealed or exposed when HIV gp120 binds to CD4[2].

Retrieved from "http://en.wikipedia.org/wiki/Cryptotope"

Appendix D

Web Results 1 - 10 of about 287,000 for **what are cryptic epitopes**. (0.30 seconds)

Detecting **Cryptic Epitopes** Created by Nanoparticles -- Lynch et al ...
Mar 21, 2006 ... These **cryptic epitopes** may trigger inappropriate cellular signaling events (as opposed to being rejected by the cells as foreign bodies). ...
stke.sciencemag.org/cgi/content/abstract/stke.3272006pe14 - Similar pages - Note this

The nature of **cryptic epitopes** within the self-antigen myelin ...
It has been proposed that such cells are specific for **epitopes** which normally are not well presented to the immune system or, in other words, are **cryptic**. ...
intimm.oxfordjournals.org/cgi/content/abstract/8/7/1035 - Similar pages - Note this

Influence of a dominant **cryptic epitope** on autoimmune T cell ...
Thus, this MBP **epitope** behaves as a classical, **cryptic epitope**2. Induction of EAE using synthetic peptides was dependent on the activation of T cells ...
www.nature.com/ni/journal/v3/n2/full/ni756.html - Similar pages - Note this

MHC class I-restricted **cryptic epitopes**: Their production and ...
MHC class I-restricted **cryptic epitopes**: Their production and immunogenicity Timothy Nicholas James Bullock, Thomas Jefferson University Date: 1996 ...
jdc.jefferson.edu/dissertations/AAI9717888/ - 10k - Cached - Similar pages - Note this

Cryptic epitopes on the nicotinic acetylcholine receptor are ...
We investigated here the presence of **cryptic** CD4+ **epitopes** on the TAChR molecule, and their relationship with potentially autoreactive CD4+ cells, ...
www.jimmunol.org/cgi/content/abstract/151/2/1025 - Similar pages - Note this

Inhibition of Experimental Metastasis by Targeting the HUIV26 ...
In previous studies, we have identified a **cryptic epitope** (HUIV26) that is specifically exposed after alterations in the triple helical structure of type IV ...
www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1606585 - Similar pages - Note this

Exposure of **cryptic epitopes** on transthyretin only in amyloid and ...
Two **cryptic epitopes** were mapped to a domain of TTR, where most mutations associated with amyloidosis occur and which we propose is displaced at the initial ...
www.pnas.org/cgi/content/abstract/96/6/3108 - Similar pages - Note this

Cryptic Epitopes on {{alpha}}-Fetoprotein Induce Spontaneous ...
Detection and characterization of antibodies to AFP **cryptic epitopes** in patient sera. Western blotting (A) and immunoprecipitation (B) analyses using n-AFP ...
cancerres.aacrjournals.org/cgi/content/full/59/21/5471 - Similar pages - Note this

The Goodpasture Autoantigen. IDENTIFICATION OF MULTIPLE **CRYPTIC** ...
Goodpasture Autoantibodies Unmask **Cryptic Epitopes** by Selectively Dissociating Autoantigen Complexes Lacking Structural Reinforcement: NOVEL MECHANISMS FOR ...
www.jbc.org/cgi/content/abstract/275/8/6030 - Similar pages - Note this

CORDIS: BIOMED 2: Projects
12. Presentation of **Cryptic Epitopes** in Autoimmune Diseases ... Our project aims to analyze the molecular mechanisms of presentation of **cryptic epitopes** and ...
cordis.europa.eu/data/PROJ_BIOMED/ACTIONeqDndSESSIONeq15033200595ndDOCeq12ndTBLeqEN_PROJ.htm - 11k - Cached - Similar pages - Note this

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